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Award Number: DAMD17-01-1-0806

TITLE: Bone Geometry as a Predictor of Tissue Fragility and Stress Fracture Risk

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REPORT DATE: October 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2002	Annual (10 Sep 01 - 10 Sep 02)

4. TITLE AND SUBTITLE
Bone Geometry as a Predictor of Tissue Fragility and Stress Fracture Risk

5. FUNDING NUMBERS
DAMD17-01-1-0806

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Having a narrow tibia relative to body mass has been shown to be a major predictor of stress fracture risk and fragility. The reason for this phenomenon is not understood. Based on studies of genetically distinct inbred mouse strains, we found a reciprocal relationship between bone mass and bone quality, such that slender bones are associated with more damageable bone tissue. *We postulate that a similar reciprocal relationship between bone mass and bone material properties exists in the human skeleton. The intriguing possibility that slender bones, like those we have demonstrated in animal models, may be composed of more damageable material than larger bones has not been considered.* To test this hypothesis, we propose to determine whether whole bone geometry is a predictor of tissue fragility in the tibia from young male donors. Tissue damageability will be assessed from biomechanical testing of compact bone samples and correlated with measures of bone slenderness. Specimens will be subjected to detailed analyses of bone microstructure, composition, and microdamage content. In the second set of experiments, these analyses will be repeated for female donors to test for gender differences in tissue fragility. Further, we will test whether fragility in cortical bone is a predictor of fragility in cancellous bone. Finally, we will conduct ultrasound measurements to identify an ultrasound parameter that is sensitive to the presence of damage and could be used for early diagnosis of stress fractures.

14. SUBJECT TERMS
stress fracture, bone mass, bone quality, biomechanics, damage, fatigue, ultrasound, non-invasive measures, genetic background

15. NUMBER OF PAGES
11

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Introduction

Having a narrow tibia relative to body mass has been shown to be a major predictor of stress fracture risk and fragility. The reason for this phenomenon is not understood. Based on studies of genetically distinct inbred mouse strains, we found a reciprocal relationship between bone mass and bone quality, such that slender bones are associated with more damageable bone tissue. *We postulate that a similar reciprocal relationship between bone mass and bone material properties exists in the human skeleton. The intriguing possibility that slender bones, like those we have demonstrated in animal models, may be composed of more damageable material than larger bones has not been considered.* To test this hypothesis, we propose to determine whether whole bone geometry is a predictor of tissue fragility in the tibia from young male donors. Tissue damageability will be assessed from biomechanical testing of compact bone samples and correlated with measures of bone slenderness. Specimens will be subjected to detailed analyses of bone microstructure, composition, and microdamage content. In the second set of experiments, these analyses will be repeated for female donors to test for gender differences in tissue fragility. Further, we will test whether fragility in cortical bone is a predictor of fragility in cancellous bone. Finally, we will conduct ultrasound measurements to identify an ultrasound parameter that is sensitive to the presence of damage and could be used for early diagnosis of stress fractures.

In addition to the primary focus of the grant outlined above, the parent grant was awarded a supplemental grant that will be used to 1) purchase a microComputed Tomography system and 2) support a graduate student to investigate the effects of mechanical loading on bone morphology and tissue fragility in inbred mouse strains.

Body

In the first year of this grant, we have purchased most of the ultrasound equipment and have secured 16 human tibia for analysis. The ultrasound equipment is currently being established under the guidance of Jonathon Kaufman, PhD and we are planning an experiment for this fall that will relate changes in the ultrasound signal to the level of induced tissue damage in human cortical bone. The human tibia have been examined for gross morphological features (length and width measurements). The collection of tibia include 10 males and 6 females. Of these, 14 are Caucasian, 1 is Hispanic, and 1 is Black. The age range is from 17 to 45 years of age (average = 33.1 years). The body height of the samples ranges from 152.4 cm to 182.9 cm (average = 173.2 cm). The body weight ranges from 57.2 kg to 136.1 kg (average = 80.4 kg). The body mass index ranges from 17.09 to 49.92 kg/m^2 (average = 26.95 kg/m^2). The length and width measures were determined to generate a gross measure of the slenderness of the bone.

When plotting the ratio of tibial width to length (Slenderness Ratio) versus body weight (Figure 1) revealed that the slenderness ratio was largely independent of body weight. Measures of slenderness were determined in both the antero-posterior (AP) and medio-lateral (ML) directions. A person with very thin bones for their body size will have a very low width/length measurement. We expected that the tibia would get proportionally wider relative to its length to accommodate increased body weight. This expectation was based on seeing an increase in cortical area to accommodate the increased body weight. However, the data so far imply that the external size of the bone does not scale with body weight. That is, individuals with larger body mass indices (larger weight relative to height) do not have proportionally larger bones to accommodate the increased weight. We are in the process of sectioning individual bones to

obtain measures of cross-sectional area and moment of inertia. These geometric measures will provide better estimates of bone slenderness that can then be related to physiological loads.

As seen in Figure 1, the majority of the samples collected so far fall within the 60-82 kg range. Within this range we observe a large degree of variability in the slenderness ratio. Our expectation is that the tibia with low slenderness ratios (i.e., small width for a given length) will also show large ash content values and, consequently, poor damage accumulation properties.

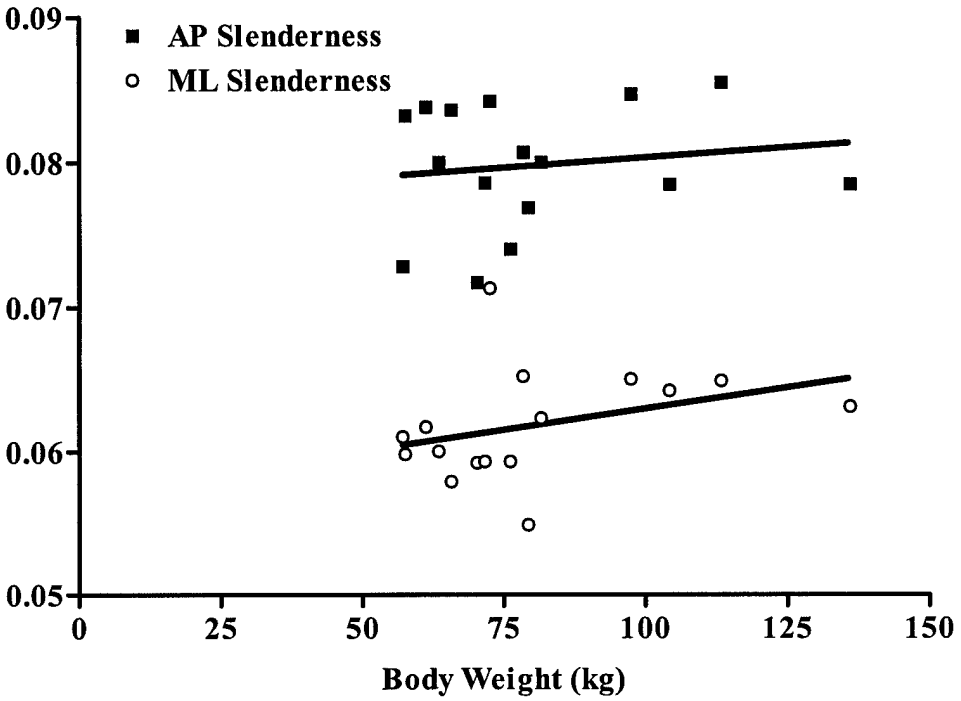


Figure 1. The slenderness of the tibia (average width/length) was plotted against body weight and revealed that slenderness does not scale with body weight.

The supplemental funding has provided new insight into the relationship between genetic background and mechanical loading (see Appendix 1). Three inbred mouse strains showed different peak whole bone mechanical properties when raised in either standard cages or large cages. Raising inbred mice in larger cages lead to a quantifiable increase in cage activity (based on a telemetric device implanted in the peritoneum of the mouse). Two of the three strains showed increased cage activity. These two strains also showed increased whole bone mechanical properties at 16 weeks of age (the time of peak bone properties). The third strain did not show increased cage activity and, consequently, did not exhibit increased bone mechanical properties. One of the interesting aspects of this data is the fact that the mice raised in the large cages had the same body weight and same muscle mass as the mice raised in the standard cages. Thus, the increased whole bone mechanical properties could not be attributed to increased body size, but to increased activity – the mice were simply more active and this lead to increased bone size. Whether the changes in bone properties are a direct result of the increased activity or secondarily associated with a change in hormone levels has yet to be determined.

The parent grant focuses on individuals with slender bones and these supplemental results provide new insight into the role that mechanical loading plays in terms of attaining high peak bone properties. Large peak bone properties are thought to lead to a reduction in skeletal

fragility later in life. Further, these results may provide new insight into the relationship between bone geometry and tissue fragility. The mice with the slender bones (AJ) was the mouse that did not show increased cage activity when placed in the larger cages. Hence, genetic background not only affects bone morphology and quality, but genetic background also affects behavioral factors that could promote increased peak bone properties.

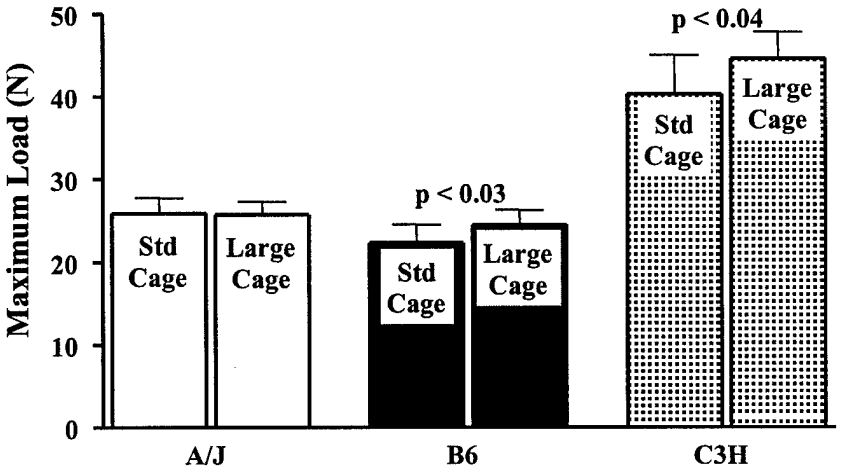


Figure 2. Maximum load of mouse femurs raised in standard (std) and large cages. (means and standard deviations) The inbred strains include A/J, C57BL/6J (B6), and C3H/HeJ (C3H). Differences within groups were determined by analysis of variance.

The supplemental funding has also provided new insight into the relationship between bone morphology and mineral content (Appendix 2). This relationship was investigated for the femurs of three inbred mouse strains using a empirical model of growth and development (Figure 3). The first 65 days of tissue age represent the completion of primary mineralization and a significant portion of secondary mineralization and therefore represent a majority of the variation in tissue ash content. If the mineralization process was identical for the three inbred strains (i.e. identical mineralization rates and maximum mineral contents), then similar distributions of tissue from 1 to 65 days of age between the strains might suggest similar ash content values at 15 weeks of age. Similar ash content values, however, are not observed (Figure 4). The lower ash content of B6 femurs did not correlate to younger tissue distributions nor did the higher ash content of A/J femurs correlate with older tissue distributions. Thus, inter-strain differences in ash content are most likely not explained by variation in tissue age distributions due to inherent differences in modeling during growth and development. These results indicate that the matrix deposition and/or mineralization process may be different for each of the inbred strains. This variation can result from either differences in the rate of mineralization or the magnitude of mineral that can be deposited within the matrix. Thus, to have a complete understanding of inter-strain variation in ash content and its effect on whole bone function a more detailed description of primary and secondary mineralization processes in mice is necessary.

These results provide new insight into the genetic complexity of the skeleton. Here, we show that in addition to genetic regulation of morphological traits, the processes controlling mineralization may also be genetically regulated. The differences in whole bone stiffness of

these three inbred strains (A/J = BL/6 < C3H), which are inversely related to peak tissue level strains, imply that there may be differences in the underlying mechanobiology. Thus, genetic variation in whole bone mechanical function may depend on the interrelationship between growth, adaptation and compositional quality.

Figure 3. Prediction of site-specific distribution of actual tissue age for femoral cross sections of three inbred (A/J, B6, C3H) mice at 15 weeks of age (1st row). Representative histological cross-sections are shown in the 2nd row.

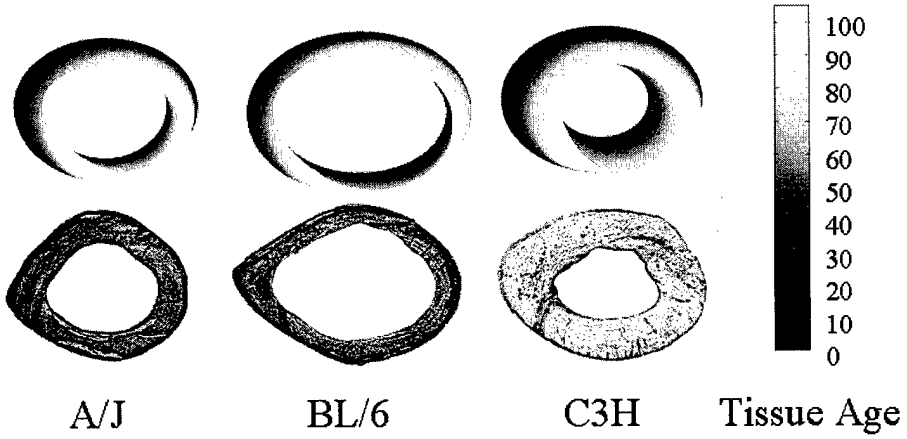
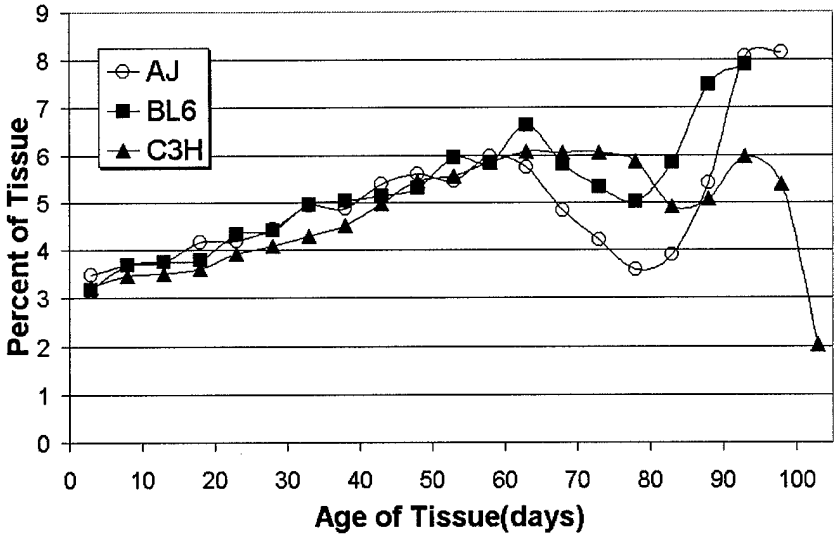


Figure 4. The area fraction of tissue at each age for three inbred mouse strains at 15 weeks of age.



Key Research Accomplishments

1. Preliminary data suggest that the external size of the bone does not scale with body weight. That is, individuals with larger body mass indices (larger weight relative to height) do not have proportionally larger bones to accommodate the increased weight.

2. Increased cage activity of inbred mice lead to increased whole bone mechanical properties. These changes in peak bone properties could not be attributed to increased body size or muscle mass.

3. Based on a mathematical model of bone growth and development, we show that in addition to genetic regulation of morphological traits, the processes controlling mineralization may also be genetically regulated.

Reportable Outcomes

Abstracts

Price C, Hernandez C, Nadeau J, Jepsen KJ. Genetic Variations in the Structural Efficiency of Long Bones May Contribute to Variations in Mineralization Among Inbred Mouse Strains. Accepted for Presentation at the American Society of Bone and Mineral Research, April, 2002.

Wolde-Semait HT, Ryan J, Jepsen KJ. The effect of genotype-environment interactions on peak bone properties. Submitted to the Orthopaedic Research Society, July, 2002. see Appendix 1.

Price C, Hernandez CJ, Jepsen, KJ. Computational model predicts genetic differences in bone tissue age and mineralization. Submitted to the Orthopaedic Research Society, July, 2002. see Appendix 2.

Funding

Additional funding has been secured through a Pilot Project program sponsored by the Howard Hughes Medical Institute. The title of this project is "A Mathematical Model of Bone Growth and Development: The Effects of Genotype-Environment Interactions on Peak Bone Mass and Strength". The goal of this proposal is to develop a mathematical model of growth and development of mouse long bone to determine why the mineral content is higher in bones that are slender relative to body size. Although this grant is focused on the mouse skeleton, we anticipate that we will obtain a general insight into the genetic and biological processes that regulate bone geometry (bone mass). This grant is for $50,000 per year for 2 years (July, 2002 to July, 2004).

Conclusions

The results to date have provided new insight into the relationship between bone morphology and tissue mechanical properties. The investigations of the mouse skeleton revealed that genetic variations in bone morphology strongly influence tissue mechanical properties through variations in matrix composition. Whether similar compositional variations are present in the human skeleton will be determined in the next year.

References

none

Appendices

Appendix 1

Wolde-Semait HT, Ryan J, Jepsen KJ. The effect of genotype-environment interactions on peak bone properties. Submitted to the Orthopaedic Research Society, July, 2002.

Appendix 2

Price C, Hernandez CJ, Jepsen, KJ. Computational model predicts genetic differences in bone tissue age and mineralization. Submitted to the Orthopaedic Research Society, July, 2002.

THE EFFECT OF GENOTYPE-ENVIRONMENT INTERACTIONS ON PEAK BONE PROPERTIES

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Introduction. Effective strategies for fracture prevention are critical for reducing the morbidity and mortality associated with osteoporosis. Many bone traits (including BMD) are known to be influenced by genetic as well as by environmental factors such as physical activity, diet, nutrition, and hormonal status. However, genotype-specific responses to environmental changes are poorly understood. Inbred mouse strains have provided an opportunity to examine these genotype-environment interactions that would otherwise be difficult to study in the human population. In mice, genotype-specific adaptations to changes in loading have only been examined in the context of forced exercise [1] and externally applied mechanical loads to adult tissue [2]. Other studies reported measurable changes in mouse bone properties following moderate changes in normal cage activity indicating that mouse bone is capable of adapting to subtle changes in environment [3]. In this study, we test whether a simple change in environment, i.e. increased cage size, will produce a self-initiated behavioral response leading to measurable changes in bone properties. To test for genetic effects, we examined adaptive responses in three inbred mouse strains (A/J, C57BL/6J, C3H/HeJ) showing significant variation in peak bone properties.

Methods. A total of 120 female A/J, C57BL/6J (B6), and C3H/HeJ (C3H) inbred mice were purchased from Jackson Laboratory and randomly split into 12 groups (3 strains x 2 environments x 2 time points, n=10/group). Mice were housed beginning at 4 weeks of age in either standard (75 sq. inches) or large (153 sq. inches) cages and then sacrificed at 8 or 16 weeks of age. All mice (5 mice/cage) were housed in the same room, fed *ad libitum*, and handled according to the institute's animal care policies.

Adaptive changes to different environments were assessed in terms of biological, mechanical, and morphometric measures. Mechanical properties (stiffness, maximum load, post-yield deflection) were determined by loading left femurs to failure in 4-point bending at 0.05 mm/sec. Dynamic and static histomorphometric measures were determined by embedding right femurs in poly-methylmethacrylate and sectioning the diaphyses transversely. Changes in the mineral apposition rate (MAR) and percent labeled surface (dLS/BS) were determined from double fluorescent labels (oxytetracycline 30mg/kg, calcein 10mg/kg) administered 8 and 2 days prior to sacrifice. Changes in mechanical and histomorphometric measures within each strain were determined by a 2-way ANOVA.

An additional group of 90 mice (3 strains x 2 environments x 3 cages x 5 mice/cage) were used to examine changes in activity due to increased cage size. A telemetric device that monitors activity and temperature (MiniMitter, Bend, OR, USA) was implanted into the peritoneal cavity of 8-wk old female mice. This particular device determines the activity of a single mouse while caged with 4 others. Transmitters have so far been implanted in 5-6 mice per strain (approx. 3 mice/cage type). Each mouse was monitored at different times post-implantation up to 18 weeks of age and the total activity count over a 24 hour period was determined by averaging over all days examined.

Results. No differences in body weight were observed between mice raised in large cages versus standard cages for all three strains indicating that this environmental change did not affect body size. At 8 weeks of age, no differences in any of the mechanical properties were observed between mice raised in standard or large cages for all three strains. By 16 weeks of age, femurs from A/J mice raised in large cages were not different from mice raised in standard cages (Fig 1). In contrast, B6 mice raised in large cages showed a 25% increase in stiffness ($p<0.004$) and a 10% increase in maximum load ($p<0.03$) compared to mice raised in standard cages. Likewise, the femurs of C3H mice raised in large cages showed a 15% increase in stiffness ($p<0.06$) and a 10% increase in maximum load ($p<0.04$) compared to mice raised in standard cages. Neither C3H nor B6 showed differences in post-yield deflection between the two cages indicating that environment did not affect bone brittleness.

Preliminary data for mouse activity revealed inter-strain differences in the response to cage size (Fig 2). A/J mice showed no change in activity when raised in the larger cage. In contrast, activity counts increased 26% in C3H mice raised in large cages compared to standard cages. The average data for B6 mice suggests there was no change in activity due to cage size. However, one of the B6 mice raised in a standard cage showed an unusually high level of activity leading to unclear preliminary results. Additional transmitter implantations are needed to establish normal intra- and inter-strain variability.

Preliminary histomorphometric analyses conducted on 8 week old B6 and C3H femurs revealed no changes in the percent surface labeled (dLS/BS) for either strain. Further, B6 femurs showed no changes in MAR on either periosteal or endosteal surfaces (data not shown). In contrast, C3H femurs raised in large cages showed a significantly greater MAR on the endosteal surface (5.4 ± 0.5microns/day vs 6.2 ± 0.7 microns/day, $p<0.04$), but not on the periosteal surface.

Discussion. The data clearly show that simply doubling available cage area results in measurable changes in whole bone mechanical properties of inbred mice. Changes in whole bone stiffness and maximum load indicate adaptive changes in underlying morphological and compositional bone traits. Evidence of adaptation was present at 8 weeks of age for C3H femurs, following only 4 weeks in the larger cage. Adaptive changes varied among inbred strains indicating that genetic background plays an important role in the response to environmental changes. Moreover, preliminary data suggest that the genotype-specific adaptive changes during growth were mediated by changes in cage activity. Previous studies have failed to explain the larger bone mass of C3H femurs compared to B6 on the basis that C3H long bone is more sensitive to mechanical loading [1,2]. Our data, measured during growth, indicate that C3H is just as responsive to changes in activity as B6.

These results have important implications for understanding how genetic background affects bone properties at the time of peak bone mass. Since more that 95% of peak bone mass is attained by late adolescence, modifying the environment during skeletal growth may have beneficial effects for increasing peak bone properties. The current data suggest that behavior during the growth phase may play a more important role in the development of peak bone properties than previously thought.

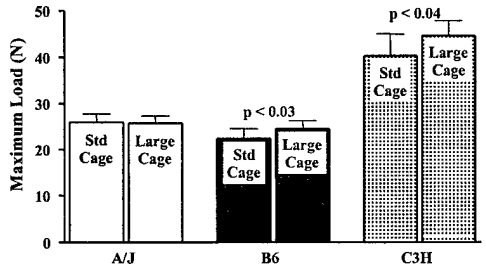


Fig 1. Maximum load of mouse femurs raised in standard (std) and large cages. (means and standard deviations)

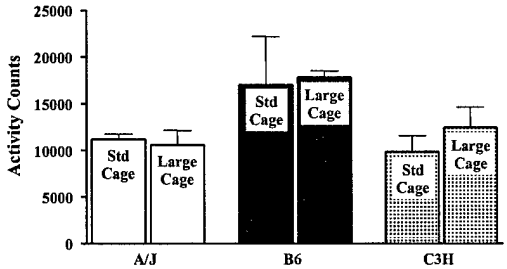


Figure 2. Average number of activity counts per 24 hour period for mice raised in standard (std) and large cages. (means and standard deviations)

Acknowledgements. NIH AR44927, DAMD17-01-1-0806.
References. [1] Kodama, *Calc Tissue Int* 66, 2000. [2] Akhter, *Calc Tissue Int* 63, 1998. [3] Gordon, *Bone* 10, 1989.

COMPUTATIONAL MODEL PREDICTS GENETIC DIFFERENCES IN BONE TISSUE AGE AND MINERALIZATION

*Price, C; *Hernandez, C J; +*Jepsen, K J
+*Leni & Peter W. May Department of Orthopaedics, Mount Sinai School of Medicine, New York, NY

Introduction. Genetic factors are generally acknowledged to contribute to skeletal fragility and osteoporotic fracture risk. However, the functional links that characterize how genotype affects phenotype (fracture risk) are not well understood. Previously we demonstrated that differences in femoral stiffness, strength, and brittleness among inbred mouse strains (e.g, A/J, C57BL/6J, C3H/HeJ) at the time of peak bone properties (15 weeks) were explained by the relative magnitude of three underlying bone traits – cortical area, moment of inertia, and ash content [1]. It was observed that morphological variations among the inbred strains were accompanied by compensatory changes in ash content suggesting that these underlying traits were related. The changes in ash content, which were positively correlated with bone stiffness and negatively correlated with brittleness, played an important role in whole bone function and, therefore, warranted further investigation.

Differences in bone modeling during growth have been observed among inbred strains [2] raising the possibility that the variation in ash content were due simply to differences in tissue age that arise from bone modeling/remodeling. To further explore the relationship between morphological and compositional bone traits and to explain observed differences in ash content among inbred strains of mice [A/J (68.5%), BL/6 (65.1%), C3H (67.4%)], we developed a computational model characterizing inter-strain differences in femoral diaphyseal growth and development. Using the computational model, we predicted the actual tissue age for these three strains of mice. Assuming that the mineralization process is identical for the three strains, then tissue age should be an indication of bone mineral content. If there were no differences in tissue age between inbred strains, this model implies that there are genetic differences in the way matrices are deposited and/or mineralized.

Methods. Male and female A/J, C57BL/6J (BL/6), and C3H/HeJ (C3H) mice were purchased from Jackson Laboratory at 6 weeks of age and bred to generate female offspring at 1, 4, 7 days, and 2, 4, 8, 15, 26 and 52-weeks of age (n = 4-15/age/strain). Mice were raised under identical conditions and handled according the institute's animal care policies. Double fluorescent labels (calcein green, 10 mg/kg; oxytetracycline, 30mg/kg) were administered to the 4, 8, 15, 26 and 52-week groups to obtain measures of mineral apposition rate (MAR). Femurs were embedded in poly-methylmethacrylate, sectioned transversely at the mid-diaphysis, and imaged to obtain periosteal and endosteal radii for each anatomic quadrant, the cortical area, and polar moment of inertia.

A computational model of bone growth was created for each inbred strain based upon measured values of bone morphology at birth, radial growth of the periosteal and endosteal surfaces, and cortical drift of the femoral diaphysis. Cortical drift (i.e. translation of the geometric centroid toward the postero-lateral quadrant) was modeled as a physiologically scaled normal distribution based on maximum MAR. The femoral cortex was modeled as a hollow ellipse. The model was validated by comparing the predicted values of cortical area, polar moment of inertia, and MAR with measured values. Tissue age was calculated (from 1 day to 1 year of age) for each strain by recording the formation and resorption of each moiety of bone in the model. The fraction of femoral cross-section with tissue age greater than the primary mineralization phase (approximately 5 days [3]) was calculated and compared between strains.

Results. The model accurately predicted values of cortical area and moment of inertia to within +5.0% of the mean values measured for each inbred strain at each time point (average absolute percent deviation). This discrepancy is consistent with that expected for the elliptical approximation of the femoral cross section (mean error of approximation of the area: A/J= +2.3%, BL/6= +6.0%, and C3H= +3.5%). Predictions of maximum MAR were also consistent with measured values.

The model predicted a non-uniform distribution of tissue age in representative cross sections for each inbred strain at 15 weeks of age (Fig 1). The actual tissue age varied from 1 to 105 days within each cross-section and these age distributions corresponded to histologically observed variation in bone microstructure. For 15-week old mice, the cross-section of each inbred strain had a similar distribution of tissue aged between 1 and 65 days (Fig. 2), well into the secondary mineralization phase. The predicted fraction of bone that had already undergone primary mineralization (i.e., >5 days old) was 96.4% for A/J, 96.3% for BL/6, 96.7% for C3H. Additionally the model predicts that the average tissue age was similar for all three strains.

Discussion. The first 65 days of tissue age represent the completion of primary mineralization and a significant portion of secondary mineralization [3] and therefore represent a majority of the variation in tissue ash content. If the mineralization process was identical for the three inbred strains (i.e. identical mineralization rates and maximum mineral contents), then similar distributions of tissue from 1 to 65 days of age between the strains might suggest similar ash content values at 15 weeks of age. Similar ash content values, however, are not observed [1]. The lower ash content of BL/6 femurs did not correlate to younger tissue distributions nor did the higher ash content of A/J femurs correlate with older tissue distributions. Thus, inter-strain differences in ash content are most likely not explained by variation in tissue age distributions due to inherent differences in modeling during growth and development [2]. These results indicate that the matrix deposition and/or mineralization process may be different for each of the inbred strains. This variation can result from either differences in the rate of mineralization or the magnitude of mineral that can be deposited within the matrix. Thus, to have a complete understanding of inter-strain variation in ash content and its effect on whole bone function a more detailed description of primary and secondary mineralization processes in mice is necessary.

These results provide new insight into the genetic complexity of the skeleton. Here, we show that in addition to genetic regulation of morphological traits [1], the processes controlling mineralization may also be genetically regulated. The differences in whole bone stiffness of these three inbred strains (A/J = BL/6 < C3H), which are inversely related to peak tissue level strains, imply that there may be differences in the underlying mechanobiology. Thus, genetic variation in whole bone mechanical function may depend on the interrelationship between growth, adaptation and compositional quality.

Figure 1. Prediction of site-specific distribution of actual tissue age for femoral cross sections of three inbred mice at 15 weeks of age (1st row). Representative histological cross-sections are shown in the 2nd row.

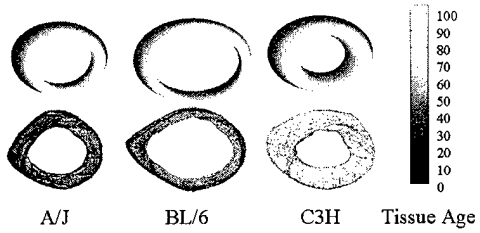
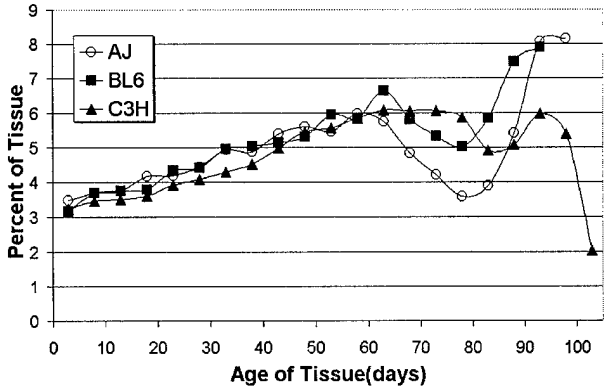


Figure 2. The area fraction of tissue at each age for three inbred mouse strains at 15 weeks of age.



References. [1] Jepsen ORS, 2001. [2] Sheng, Bone 25, 1999 [3] Parfitt, in *Bone Histomorphometry: Techniques and Interpretation* 1983.
Acknowledgements. NIH AR44927, DAMD17-01-1-0806.